Liver Fibrosis and Metabolic Alterations in Adults With alpha-1-antitrypsin Deficiency Caused by the Pi*ZZ Mutation

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Abstract: BACKGROUND AIMS Alpha-1 antitrypsin deficiency (AATD) is among the most common genetic disorders. Severe AATD is caused by a homozygous mutation in the SERPINA1 gene that encodes the Glu342Lys substitution (called the Pi*Z mutation, Pi*ZZ genotype). Pi*ZZ carriers may develop lung and liver diseases. Mutation-associated lung disorders have been well studied, but less is known about the effects in liver. We assessed the liver disease burden and associated features in adults with this form of AATD. METHODS We collected data from 554 Pi*ZZ adults (403 in an exploratory cohort, 151 in a confirmatory cohort), in 9 European countries, with AATD who were homozygous for the Pi*Z mutation, and 234 adults without the Pi*Z mutation (controls), all without pre-existing liver disease. We collected data on demographic parameters, comorbidities, lung- and liver-related health, and blood samples for laboratory analysis. Liver fibrosis was assessed non-invasively via the serum tests Aspartate Aminotransferase to Platelet Ratio Index and HepaScore and via transient elastography. Liver steatosis was determined via transient elastography-based controlled attenuation parameter. We performed histologic analyses of livers from transgenic mice that overexpress the AATD-associated Pi*Z variant. RESULTS Serum levels of liver enzymes were significantly higher in Pi*ZZ carriers vs controls. Based on non-invasive tests for liver fibrosis, significant fibrosis was suspected in 20%-36% of Pi*ZZ carriers, whereas signs of advanced fibrosis were 9- to 20-fold more common in Pi*ZZ carriers compared to non-carriers. Male sex; age older than 50 years; increased levels of alanine aminotransferase, aspartate aminotransferase, or -glutamyl transferase; and low numbers of platelets were associated with higher liver fibrosis burden. We did not find evidence for a relationship between lung function and liver fibrosis. Controlled attenuation parameter 280 dB/m, suggesting severe steatosis, was detected in 39% of Pi*ZZ carriers vs 31% of controls. Carriers of Pi*ZZ had lower serum concentrations of triglyceride and low- and very-low-density lipoprotein cholesterol than controls, suggesting impaired hepatic secretion of lipid. Livers from Pi*Z-overexpressing mice had steatosis and down-regulation of genes involved in lipid secretion. CONCLUSIONS In studies of AATD adults with the Pi*ZZ mutation, and of Pi*Z-overexpressing mice, we found evidence of liver steatosis and impaired lipid secretion. We identified factors associated with significant liver fibrosis in patients, which could facilitate hepatologic assessment and counseling of individuals who carry the Pi*ZZ mutation. ClinicalTrials.gov Number NCT02929940.

DOI: https://doi.org/10.1053/j.gastro.2019.05.013

Journal Article
Accepted Version

Originally published at:
Hamesch, Karim; Mandorfer, Mattias; Pereira, Vítor M; Moeller, Linda S; Pons, Monica; Dolman, Grace E; Reichert, Matthias C; Schneider, Carolin V; Woditsch, Vivien; Voss, Jessica; Lindhauer, Cecilia; Fromme, Malin; Spivak, Igor; Guldiken, Nurdan; Zhou, Biaohuan; Arslanow, Anita; Schaefer, Benedikt; Zoller, Heinz; Aigner, Elmar; Reiberger, Thomas; Wetzel, Martin; Siegmund, Britta; Simões, Carolina; Gaspar, Rui; Maia, Luís; Costa, Dalila; Bento-Miranda, Mário; van Helden, Josef; Yagmur, Eray; Bzdok, Danilo; Stickel, Felix (2019). Liver Fibrosis and Metabolic Alterations in Adults With alpha-1-antitrypsin Deficiency Caused by the Pi*ZZ Mutation. Gastroenterology, 157(3):705-719.e18.
DOI: https://doi.org/10.1053/j.gastro.2019.05.013

Rapid Response to Treatment of Autoimmune Hepatitis Associated with Remission at 6 and 12 Months

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Grant support: MP was supported by the Janos Bolyai Research Scholarship of the Hungarian Academy of Sciences (BO/00232/17/5) and the New National Excellence Program of the Ministry of Human Capacities (ÚNKP-19-4 Bolyai Plus).

Disclosures: All authors report no potential conflicts that are relevant to the manuscript.

Writing assistance: None.

Author contributions: MAH conceived the study, provided data and supervised the study. TG and JD supervised the study. SP, JV, BH, GB, CN, JH, RT, EJ, MM, MP, NS, FS, CE, EO, TP, FN, DK, AK, HW, CS and AL provided and collected data. SP and TG analyzed the data. SP wrote the manuscript. All authors critically reviewed the manuscript and provided the final version.

Tables and figures:

5 tables / 1 figure

Word count

Abstract: 260

Total manuscript: 3949

Abbreviations:

AIH, autoimmune hepatitis; ALT, alanine aminotransferase; AS-AIH, acute-severe autoimmune hepatitis; AST, aspartate aminotransferase; IAIHG, international autoimmune hepatitis group; HR, hazard ratio; IgG, immunoglobulin G; OR, odds ratio; ULN, upper limit of normal

ABSTRACT

Background & Aims: Changes in serum levels of transaminases immediately after initiation of treatment for autoimmune hepatitis (AIH) might be associated with biochemical markers of remission and liver-related events. We assessed the outcomes of patients with vs without rapid responses to treatment of AIH in a large international cohort.

Methods: We performed a retrospective cohort study, collecting data from 2 independent cohorts of adults with AIH from 12 centers in 7 countries in Europe. We collected information on patient demographics; serologic, histologic, and biochemical analyses; and treatment. We used a receiver operating characteristic curve and Youden index to calculate the optimal percentage decrease in level of aspartate aminotransferase (AST) after 8 weeks of treatment that associated with normalization of transaminase levels after 26 weeks of treatment with predniso(lo)ne (primary outcome) in the first (discovery) cohort (n = 370). We evaluated the results in the second (validation) cohort (n = 370). Secondary outcomes were liver-related death or transplantation. We performed univariate and multivariable logistic and Cox regression with correction for confounders.

Results: A significant decrease in level of AST after 8 weeks of treatment was significantly associated with normalization of transaminase levels at 26 and 52 weeks (P<.001); a decrease of more than 80% in level of AST was associated with optimal normalization. In both cohorts, rapid responders (≥80% decrease in level of AST after 8 weeks) were more likely to achieve normalization of transaminases at 26 and 52 weeks when compared to non-rapid responders. Rapid responders in the discovery cohort had lower risk of liver-related death or transplantation (adjusted hazard ratio 0.18; 95% CI 0.05–0.63; P=.007), although this was not confirmed in the validation

cohort. Results from measurement of alanine aminotransferase did not differ significantly from those of AST for the primary outcome. Slow responders (without normalization of transaminases after 1 year) had the highest risk of liver transplantation or liver-related death.

Conclusions: In a retrospective study of patients with AIH, we found that a rapid response to treatment, based on level of AST after 8 weeks, associates with normalization of transaminase levels in the following year. Patients with a rapid response also have a lower risk of liver-related death or transplantation than patients without this rapid response.

Keywords

Induction therapy, prognostic factor, liver enzyme, steroid

INTRODUCTION

Autoimmune hepatitis (AIH) is a rare, chronic liver disease that is characterized by elevated serum transaminases and immunoglobulin G (IgG), inflammatory liver histology and presence of circulating auto antibodies [1, 2]. Treatment consists of induction therapy with corticosteroids followed by maintenance therapy with azathioprine (AZA) [3]. Biochemical remission is defined as normalization of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and IgG below the upper limit of normal (ULN). This endpoint is associated with low histological disease activity and regression of fibrosis [4].

Reports on the relationship between elevation and dynamics of transaminases and relevant outcomes are limited. Cases with AST levels greater than 10 times ULN at presentation have a lower risk of developing cirrhosis and had a better long-term outcome [5]. Patients who do not achieve at least a 50% decrease of transaminases within six months run an increased risk for liver transplantation [6].

A large proportion of AIH patients have a rapid decline of serum transaminases in the first weeks after initiation of steroids, persisting throughout treatment. The exact relationship between the rapidity of decline in transaminases during treatment and long-term clinical events, such as liver transplantation and liver-related mortality, is unknown. We hypothesized that patients with a rapid decrease in transaminases have a higher probability to achieve biochemical remission and a lower risk for liver-related morbidity later in time. Therefore, we used data from two independent cohorts of AIH patients to investigate the relationship between early treatment response and the effect on biochemical remission, liver related mortality, and liver transplantation.

METHODS

Study design

We performed a retrospective cohort study, establishing two independent cohorts of AIH patients from 12 centers across seven countries in Europe. We included patients with a probable or definite AIH diagnosis according to the simplified International Autoimmune Hepatitis Group (IAIHG) score [7-9]. When patients scored as 'No AIH' by the simplified criteria, but were treated by their physicians as AIH patients, the revised score was used to calculate a score per patient. Only patients with a pre-treatment score >10 were classified as AIH and were included in our study. Only patients who were ≥18 years old at time of diagnosis were included. We excluded patients who had variant syndromes with primary biliary cholangitis, primary sclerosing cholangitis, or other forms of liver disease such as viral hepatitis and non-alcoholic steatohepatitis. Ethics approval was waived after review by local institutional review board.

Data collection

Patient data were retrieved from patients records and local databases. We collected demographics variables, serological, histological, biochemical and treatment variables. The original pathology report was used to classify a patient as cirrhotic. Additionally, data on mortality and liver transplantation was collected. Laboratory values were collected at baseline and after 8, 26 and 52 weeks of therapy.

Outcomes

Primary outcomes were normalization of transaminases after 26 and 52 weeks of treatment. The gender specific ULN for ALT and AST of each center was used. Secondary endpoints included biochemical remission, defined as normalization of transaminases and normal serum IgG after 26 and 52 weeks of treatment, liver related

mortality or liver transplantation, all-cause mortality and development of hepatocellular carcinoma (HCC). In case of missing ALT or AST values at the 26 or 52 week time point, we used last observation carried forward to account for missing values.

Analysis

We randomly generated a discovery and validation cohort with stratification for baseline predniso(lo)ne dose. First, we analyzed the correlation between percentage decrease of AST after 8 weeks and normalization of transaminases after 26 weeks in the discovery cohort (n = 370) using a receiver operating characteristic (ROC) curve analysis. The Youden index was calculated to generate a cut-off level of percentage of AST decrease after 8 weeks.

Second, patients from the discovery cohort were divided into two groups based on the cut-off, generated from the first analysis. Patients with a percentage fall of AST after 8 weeks above the cut-off were classified as 'rapid responders' and were compared to patients with a fall of AST below the cut-off. Univariate comparisons between the two groups were made with the chi-square test, Mann-Whitney U test or t-test as appropriate. We applied logistic regression in order to determine endpoints in the two groups of patients. The final regression model included institute, cirrhosis, acute-severe AIH (AS-AIH defined as a presentation with an international normalized ratio ≥1.5 without evidence of cirrhosis [10]), predniso(lo)ne dose, use of maintenance therapy, AST at baseline and bilirubin at baseline. Results of the multivariable logistic regression are presented as odds ratio's (OR) and 95% confidence intervals (CI). We used Kaplan-Meier curves with Log-rank testing and Cox regression with adjustment for confounders for the composite endpoint of liver related mortality and

transplantation. Results of the Cox regression analysis are presented as hazard ratio's (HR) with 95% CIs.

Third, the cut-off generated from the discovery cohort was used to determine outcomes in the validation cohort (n = 370). We performed similar univariate and multivariable analyses as in the discovery cohort. Fourth, we performed a subgroup analysis in both cohorts combined on patients with cirrhosis at presentation. Additionally, we performed exploratory analyses targeting slow responders who achieved normalization of transaminases at week 52. Analysis for the primary and secondary outcomes were also performed with percentage ALT decrease as the independent variable in both cohorts. P-values <0.05 were considered statistically significant. Statistical analysis was performed using SPSS version 25 (IBM Corporation, Armonk, NY, USA).

RESULTS

Population

Both the discovery and validation cohort consisted of 370 patients. Most patients were female (74.5%). Patients in the validation cohort were slightly older at time of diagnosis compared to patients in the discovery cohort (49.58 years vs. 47.09 years, p = 0.04). Other baseline and treatment characteristics were comparable between the cohorts (supplementary table 1).

ROC analysis

Percentage decrease of AST after 8 weeks of treatment was significantly associated with normalization of transaminases at 26 weeks of treatment in ROC analysis (area under the curve 0.65, 95% CI 0.59 – 0.71, p < 0.001). The highest

Youden index was 0.274, which corresponded with an AST decrease of 80%. This percentage was used as cut-off to determine patients with a rapid treatment response. Corresponding sensitivity, specificity, and positive predictive value were 64.6%, 62.8% and 77.3% respectively.

Discovery cohort: baseline characteristics

Of all patients in the discovery cohort, 60.8% (225/370) of patients were scored as rapid treatment responders (≥80% AST decrease after 8 weeks). Rapid responders had significantly higher transaminases (ALT x ULN 21.34 vs. 3.27, $p < 0.001$; AST x ULN 19.29 vs. 2.61, $p < 0.001$) and total bilirubin (107 vs. 21 µmol/l, $p < 0.001$) levels at baseline when compared to patients without a rapid treatment response (table 1). Rapid responders were less likely to have cirrhosis at baseline when compared to slow responders (13.8% vs. 24.1%, $p = 0.01$) and were more likely to have AS-AIH (21.8% vs. 6.9%, $p < 0.001$). Patients with a rapid treatment response were treated with higher initial predniso(lo)ne dosages (0.73 mg/kg/day vs. 0.50 mg/kg/day, $p < 0.001$).

Discovery cohort: outcomes

Rapid responders had higher rates of normalization of transaminases at 26 weeks compared to patients with a slower treatment response: 77.3% vs. 57.2% ($p < 0.001$). This difference persisted after 52 weeks of treatment: 86.7% of rapid responders had normalization of transaminases vs. 64.8% of non-rapid responders ($p < 0.001$). In a subgroup of patients with available IgG ($n = 227$ for 26 weeks, $n = 210$ for 52 weeks), we found that biochemical remission rates were higher in the rapid responders when compared to non-rapid responders: 77.1% vs. 52.9% ($p < 0.001$) and 79.7% vs. 61.2% ($p = 0.004$) or 26 and 52 weeks respectively (table 2).

Multivariable logistic regression showed that rapid responders had a higher probability to reach normalization of transaminases after 26 weeks (OR 3.63, 95% CI 1.94 – 6.79, p < 0.001) and 52 weeks (OR 4.99 95% CI 2.44 – 10.24, p < 0.001) of treatment. The same results were observed for biochemical remission in patients with available IgG: ORs were 4.51 (95% CI: 2.05 – 9.92, p < 0.001) and 2.77 (95% CI 1.18 – 6.47, p = 0.02) for 26 and 52 weeks respectively (table 3). A sensitivity analysis in a dataset without imputed AST and ALT values we found similar significant results (p < 0.001 for normalization of transaminases at both 26 and 52 weeks).

To exclude the presence of cirrhosis as a confounder, we performed a sensitivity analysis in patients without cirrhosis at time of diagnosis in the discovery cohort, which demonstrated consistency with our primary analysis. Non-cirrhotics rapid responders had a higher chance of normalization of transaminases at 26 and 52 weeks of treatment when compared to non-cirrhotics who responded slower (OR 3.62 95% CI 1.81 – 7.26, p < 0.001 and OR 4.18 95% CI 1.87 – 9.36, p = 0.001 for week 26 and 52 respectively).

During a median follow-up of 6.2 years, liver related death or liver transplantation, as a composite endpoint, occurred less frequently in rapid responders: 3.1 vs. 15.9% (Log rank p < 0.001) (figure 1). This also held for all-cause mortality which occurred in 4.9% of rapid responders vs. 13.9% in slow responders (Log rank p = 0.001). Multivariable Cox-regression showed that rapid responders were at a lower risk of liver related death or transplantation (adjusted HR 0.18 95% CI 0.05 – 0.63, p = 0.007) and for all-cause mortality, (adjusted HR 0.26 95% CI 0.09 – 0.75, p = 0.01). Development of HCC occurred only in the slow responder group (2.8% vs. 0%, p = 0.01).

Slow responders in the discovery cohort who did not achieve normalization of transaminases after 52 weeks of treatment had higher rates of liver related death or liver transplantation when compared to slow responders that did achieve normalization of transaminases after 52 weeks: 35.3% vs. 5.3% ($p < 0.001$). This difference remained significant after multivariable Cox regression (adjusted HR 0.13 95% Ci 0.03 – 0.52, $p = 0.004$). Slow responders who did not normal transaminases after 52 weeks had higher pre-treatment transaminases and bilirubin and were more frequently cirrhotic (supplementary table 7). Similar observations were made in the validation cohort: slow responders that did not reach normal transaminases after 52 weeks had numerically higher rates of liver related death or liver transplantation, although this did not reach statistical significance: 16.0% vs. 8.4% ($p = 0.17$). However, when corrected for confounders in the multivariate Cox-regression, reaching normal transaminases at week 52 predicted a lower chance on liver related death or transplantation (adjusted HR 0.27 95% CI 0.08 – 0.84, $p = 0.02$).

Validation cohort: baseline characteristics

In the validation cohort, 60.8% (225/370) patients were assigned to the rapid responder group. We observed similar differences in baseline characteristics as in the discovery cohort: rapid responders had higher ALT, AST, bilirubin and IgG at baseline (supplementary table 2). Cirrhosis was unevenly but not significantly distributed among rapid and slow responders (14.7% vs. 20.7%, $p = 0.13$). AS-AIH occurred in rapid responders (13.7%) and slow responders ((8.3%), $p = 0.11$). Rapid responders were treated with significantly higher predniso(lo)ne dosages when compared to slow responders (0.71 mg/kg/day vs. 0.51 mg/kg/day, $p < 0.001$), while use of maintenance therapy at week 26 did not differ (73.3% vs. 69.0%, $p = 0.36$).

Validation cohort: outcomes

Consistent with the results in the discovery cohort, we found that rapid responders were more likely to achieve normalization of transaminases after 26 and 52 weeks of treatment (73.3% vs. 51.7%, $p < 0.001$ and 83.6% vs. 65.5%, $p < 0.001$). The observation was made in patients with available IgG for biochemical remission after 26 and 52 weeks (72.8% vs. 42.0%, $p < 0.001$ and 84.4% vs. 56.8%, $p < 0.001$) (table 4).

Multivariable logistic regression showed a significant advantage for rapid responders for normalization of transaminases after 26 and 52 weeks (OR 2.97 95% CI 1.66 – 5.33, $p < 0.001$ and OR 2.45 95% CI 1.28 – 4.69, $p = 0.007$), or for biochemical remission at the same time points (OR 3.62 95% CI 1.68 – 7.82, $p = 0.001$ and OR 4.34 95% CI 1.77 – 10.65, $p = 0.001$) (table 5).

During a median follow-up of 6.2 years, liver related death or liver transplantation occurred more frequently in slow responders: 11.0% vs. 3.6% (Log rank $p = 0.006$) (supplementary figure 1). A multivariable Cox regression failed to assign statistical significance (adjusted HR 0.47 95% CI 0.16 – 1.39, $p = 0.17$). Similar results were seen for all-cause mortality (9.0% vs. 4.9%, Log rank $p = 0.14$; adjusted HR 0.68 95% CI 0.26 – 1.79, $p = 0.15$).

Sensitivity analysis with ALT

We performed a sensitivity analysis in patients with available ALT at week 8 in the discovery cohort (n = 326) as well as the validation cohort (n = 337). In both cohorts we found that rapid responders based on ALT had a higher likelihood for normalization of transaminases and biochemical remission when compared to slow responders. The composite endpoint of liver transplantation and liver related death was significant in

the discovery cohort, but not in the validation cohort after Cox-regression (supplementary tables 4 and 5).

Subgroup analysis in patients with cirrhosis

We performed a subgroup analysis in patients from both cohorts combined who had cirrhosis at presentation (supplementary table 3). Sixty-four (49.6%) were rapid responders. In rapid responding cirrhotics, rates of normalization of transaminases were higher than in those with a slow response: 73.4% vs. 44.6% ($p = 0.001$) and 79.7% vs. 47.7% ($p < 0.001$) for 26 weeks and 52 weeks respectively, which remained significant in the multivariable analysis (OR 8.93, 95% CI 2.69 – 29.69, $p < 0.001$; OR 5.95, 95% CI 1.92 – 18.50, $p = 0.002$). Rates of biochemical remission were also higher in the univariate analysis in rapid responding cirrhotics: 70% vs. 40% ($p = 0.009$) and 77.5% vs. 48.5% ($p = 0.01$) for 26 weeks and 52 weeks respectively. Only the 26 week time point remained significant in the multivariable analysis ($p = 0.04$ and $p = 0.42$ for week 26 and 52 respectively). The composite endpoint of liver related death or transplantation occurred less often in rapid responding cirrhotics: 6.3% vs. 27.7% ($p = 0.001$), although there was no significant difference in the multivariable analysis ($p = 0.13$).

DISCUSSION

We show that AIH patients with a substantial decrease (≥80%) of transaminases in the first 8 weeks of treatment have a high chance of normalization of transaminases and biochemical remission after 26 and 52 weeks of treatment. A rapid treatment response was independently associated with a lower risk of liver related death or liver transplantation, although only in the discovery cohort. The clinical consequence of these observations is that ≥80% decrease in transaminase levels within 8 weeks of

treatment initiation may serve as a predictor of long-term disease course and serves as a clinical tool for patient stratification. Additionally, we found that AIH patients with a slow treatment response who failed to reach normalization of transaminases after one year of treatment were at the highest risk for development of liver-related mortality or transplantation.

High transaminases were associated with a rapid response and it is possible these patients with acute AIH, are more susceptible to immunosuppressive treatment. Lower baseline transaminases in the slow responders might suggest a subclinical or protracted disease course in the months/years preceding diagnosis, leading to a delay in initiation of effective therapy, and poorer response rates [11]. Theoretically, a rapid and intense suppression of the inflammatory response may lead to hepatic stellate cell deactivation, cease proliferation of myelofibroblasts and prevent fibrosis development [12].

Earlier studies on predictors of treatment response showed that a diagnosis <18 years old, histological cirrhosis and positive soluble liver antigen/liver pancreas antigen were associated with poor short- and long term outcome [13]. However, the concept of rapidity of treatment response and its consequences has been largely unexplored. Results of our study accord with those of The King's College group who described an association between baseline AST levels and cirrhosis development as well as long-term outcome [5]. Patients with AST levels at diagnosis less than 10 times ULN had a higher risk on liver transplantation or death. Similarly, patients had higher bilirubin levels and less cirrhosis at time of diagnosis. Our study provides new and important clinical information as we show that transaminase dynamics surpass a single AST measurement as a predictor for outcomes in AIH. To further illustrate this, we stratified patients from both cohorts combined according to their initial AST elevation, which

shows that a rapid response has predictive value, regardless of baseline AST level. (supplementary table 6). Our data accord with another, much smaller study that demonstrated that a rapid treatment response (defined as a response within six months after treatment initiation) mitigated the risk for the development of cirrhosis and need for liver transplantation [14].

We found that there is a wide phenotypical heterogeneity of AIH, in terms of variation of transaminases at presentation. Slow responders had lower transaminases at baseline and AIH cirrhotics have lower transaminases on presentation [15] [16]. However, our sensitivity analysis in non-cirrhotics gave similar results, excluding cirrhosis as a confounding factor. Moreover, a rapid treatment response after 8 weeks of treatment is associated with improved outcomes regardless of presence of cirrhosis. These findings indicate that even in cirrhotics, rapidity of treatment response acts as a prognostic factor, although the statistical power precludes to confirm the benefit for our composite outcome of liver related death or transplantation.

Our study comes with limitations. First, due to its retrospective design, it carries selection bias. Only patients who had available serum transaminases in the first weeks of treatment were included in this study. However, the large number of participating centers allows us to collect a large AIH cohort that reflects real-world practice. Second, rapid responders were treated with higher predniso(lo)ne dosages, suggesting that steroid dose might act as a confounder. Indeed, we noticed that, in a subgroup of patients with available data, cumulative steroid dosages in the first year of treatment were slightly lower in slow responders, although this was not statistically significant. However, we have shown previously that initial steroid dose is independent from the likelihood of biochemical remission [17, 18]. We adjusted for steroid dose in the logistic regression model, which gave similar results to the univariate analysis. Third, we used

normalization of transaminases as our primary endpoint, which contrasts international guidelines that state that complete biochemical remission, including normalization of IgG, should be the desired endpoint in AIH [3, 19]. We found that IgG levels were not as frequently measured as guidelines stipulate. This suggests decision making in routine care is based on transaminases alone. Therefore, we chose to incorporate biochemical remission as a secondary endpoint for patients with an available IgG at the time points of interest, which yield similar. Fourth, we do not provide data on histological disease activity at diagnosis or follow-up. Very few centers use the hepatitis activity index in their histology reports. Practical and logistic hurdles hampered us from revising all the biopsy samples from the patients in our cohort. Although older studies questioned the relationship between normalization of serum markers and complete histological remission [20, 21], a recent study confirmed that biochemical remission is associated with histological disease activity and regression of fibrosis [4] . Fifth, the concept of rapid treatment response described in this study is only applicable to patients that reach the 8 week time point. Our model is of little value for AIH patients who present with acute liver failure that warrants immediate escalation of therapy or transplantation as they were excluded from our study. Sixth, we included patients with AS-AIH in both our cohorts and included them in our primary analysis. Although a subgroup analysis without AS-AIH showed similar results (data not shown), one could consider to exclude AS-AIH patients in future studies, given the differences in presentation, kinetics and prognosis. Lastly, missing data points hampered us from investigating other predictive models that had a slightly better performance than a model with AST only. Additionally, we were unable to analyse various factors that might have played a role in treatment outcomes, such as compliance to therapy, flares of AIH and drug levels. Future studies

should prospectively validate models that include a combination of ALT, AST and bilirubin after 8 weeks of treatment.

The results of our study underline that a rapid and substantial amelioration of biochemical inflammatory activity is an important prognostic factor for remission of AIH. The absence of such a response after 8 weeks might be used to identify patients that might benefit from intensified monitoring and escalation of treatment, although this hypothesis needs future prospective research. Since we observed that a large proportion of patients without a rapid response ultimately achieved biochemical remission, clinicians should be encouraged to continue adequate immunosuppression with the goal of later remission. In clinical practice, AIH patients should be monitored intensively during the first weeks after initiation of treatment, in order to obtain a clear picture of dynamics of transaminases.

To conclude, AIH patients with a rapid treatment response after 8 weeks of treatment have a favorable disease course with a high likelihood of biochemical remission and possibly a reduced risk for liver related mortality and liver transplantation. Moreover, these results suggest that stratification according to early treatment response may also identify patients at greatest risk and need for treatment intensification.

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Tables & Figures

Table 1

	<80% AST decrease at week 8 n = 145	≥80% AST decrease at week 8 n = 225	p-value
Female sex, n (%)	105 (72.4%)	171 (76%)	0.44
Age at diagnosis, year (SD)	48.08 (16.39)	46.46 (16.07)	0.35
Probable AIH diagnosis, n (%)	66 (45.5%)	100 (44.4%)	0.84
Definite AIH diagnosis, n (%)	79 (54.4%)	125 (55.6%)	0.84
ALT x ULN, median (IQR)	3.27 (5.59)	21.34 (28.31)	<0.001
AST x ULN, median (IQR)	2.61 (4.16)	19.29 (22.70)	<0.001
Bilirubin (µmol/l), median (IQR)	21 (37.5)	107 (207.1)	<0.001
INR, median (IQR)†	1.10 (0.29)	1.25 (0.54)	<0.001
IgG (g/l), median (IQR)	17.73 (13.2)	20.0 (11.7)	0.07
Cirrhosis, n (%)	35 (24.1%)	31 (13.8%)	0.01
AS-AIH, n (%)	10 (6.9%)	49 (21.8%)	<0.001
Treatment characteristics			
Prednisone dose at start (mg/kg), median (IQR)	0.50 (0.44)	0.73 (0.81)	<0.001
Predniso(lo)ne dose at start mg/day), median (IQR)	40 (40)	50 (68)	<0.001
On predniso(lo)ne at 26 weeks, n (%)	96 (96.0%)	149 (94.3%)	0.54
Predniso(lo)ne dose at 26 weeks (mg/day), median (IQR)	7.5 (10.0)	7.5 (5.0)	0.04
On predniso(lo)ne at 52 weeks, n (%)	62 (82.7%)	113 (82.5%)	0.97
Predniso(lo)ne dose at 52 weeks (mg/day), median (IQR)	5.0 (7.5)	5.0 (5.0)	0.46
Use of maintenance therapy at week 26, n (%)	105 (72.4%)	173 (76.9%)	0.33
AZA, n (%)	86 (59.3%)	148 (65.8%)	0.21
MMF, n (%)	4 (2.8%)	6 (2.7%)	0.96
TAC, n (%)	1 (0.7%)	2 (0.9%)	0.84
6-MP, n (%)	7 (4.8%)	8 (3.6%)	0.55

6-TG, n (%)	0	1 (0.4%)	0.42
CsA, n (%)	3 (2.1%)	4 (1.8%)	0.84
Other, n (%)	1 (0.7%)	3 (1.3%)	0.56

Baseline and treatment characteristics of the discovery cohort. Patients are divided into two groups based on their treatment response. † available for 291 patients. 6-MP, 6-mercaptopurine; 6-TG, 6-tioguanine; ALT, alanine aminotransferase; AS-AIH, acute-severe autoimmune hepatitis; AST, aspartate aminotransferase; AZA, azathioprine; CsA, cyclosporine; IAIHG, International Autoimmune Hepatitis Group; IgG, immunoglobulin G, IQR, interquartile range; MMF, mycophenolate mofetil; SD, standard deviation; TAC, tacrolimus; ULN, upper limit of normal.

Table 2

	<80% AST decrease at week 8 n = 145	≥80% AST decrease at week 8 n = 225	p-value
Normal transaminases at 26 weeks, n (%)	83 (57.2%)	174 (77.3%)	<0.001
Biochemical remission at 26 weeks, n (%)†	46 (52.9%)	108 (77.1%)	<0.001
Predniso(lo)ne dose ≤10 mg at 26 weeks, n (%)	32 (22.1%)	77 (34.2%)	0.01
Normal transaminases at 52 weeks, n (%)	94 (64.8%)	195 (86.7%)	<0.001
Biochemical remission at 52 weeks, n (%)¶	41 (61.2%)	114 (79.7%)	0.004
Predniso(lo)ne dose ≤10 mg at 52 weeks, n (%)	65 (44.8%)	125 (55.6%)	0.04
Liver related death or LTx, n (%)	23 (15.9%)	7 (3.1%)	<0.001
All cause mortality, n (%)	20 (13.9%)	11 (4.9%)	0.002
HCC development, n (%)	4 (2.8%)	0	0.01

Outcomes in the discovery cohort: patients with a rapid treatment response (>80% AST decrease after 8 weeks of treatment) vs. patients without a rapid treatment response. Primary outcome was normalization of transaminases after 26 and 52 weeks of treatment. For patients with available IgG, we performed a subgroup analysis for biochemical remission. Data available for: † 227 patients; ¶ 210 patients. AST, aspartate aminotransferase; HCC, hepatocellular carcinoma; LTx, liver transplantation.

Table 3

Logistic regression	Uncorrected OR	p-value	Corrected OR	p-value
Normal transaminases at 26 weeks	2.55 (1.62 – 4.01)	<0.001	3.63 (1.94 – 6.79)	<0.001
Biochemical remission at 26 weeks	3.01 (1.69 – 5.36)	<0.001	4.51 (2.05 – 9.92)	<0.001
Normal transaminases at 52 weeks	3.53 (2.11 – 5.90)	<0.001	4.99 (2.44 – 10.24)	<0.001
Biochemical remission at 52 weeks	2.49 (1.32 – 4.72)	0.005	2.77 (1.18 – 6.47)	0.02
Cox regression	**Uncorrected HR**	**p-value**	**Corrected HR**	**p-value**
Liver related death or LTx	0.18 (0.08 – 0.43)	<0.001	0.18 (0.05 – 0.63)	0.007
All cause mortality	0.32 (0.15 – 0.67)	0.002	0.26 (0.09 – 0.75)	0.01

Results of the discovery cohort after multivariable logistic regression and Cox regression for patients with a ≥80% AST decrease after 8 weeks of treatment. In all multivariable analyses we adjusted for institute, cirrhosis, AS-AIH, predniso(lo)ne dose, use of maintenance therapy, AST at baseline and bilirubin at baseline. AS-AIH, acute-severe AIH; AST, aspartate aminotransferase; HR, hazard ratio; OR, odds ratio; LTx, liver transplantation.

Table 4

	<80% AST decrease at week 8 n = 145	≥80% AST decrease at week 8 n = 225	p-value
Normal transaminases at 26 weeks, n (%)	75 (51.7%)	165 (73.3%)	<0.001
Biochemical remission at 26 weeks, n (%)†	34 (42.0%)	99 (72.8%)	<0.001
Predniso(lo)ne dose ≤10 mg at 26 weeks, n (%)	33 (22.8%)	71 (31.6%)	0.07
Normal transaminases at 52 weeks, n (%)	95 (65.5%)	188 (83.6%)	<0.001
Biochemical remission at 52 weeks, n (%)¶	50 (56.8%)	114 (84.4%)	<0.001
Predniso(lo)ne dose ≤10 mg at 52 weeks, n (%)	67 (46.2%)	135 (60.0%)	0.009
Liver related death or LTx, n (%)	16 (11.0%)	8 (3.6%)	0.004
All cause mortality, n (%)	13 (9.0%)	11 (4.9%)	0.12
HCC development, n (%)	0	0	-

Outcomes in the validation cohort: patients with a rapid treatment response (>80% AST decrease after 8 weeks of treatment) vs. patients without a rapid treatment response. Primary outcome was normalization of transaminases after 26 and 52 weeks of treatment For patients with available IgG, we performed a subgroup analysis for biochemical remission. Data available for: † 217 patients; ¶ 229 patients. AST, aspartate aminotransferase; HCC, hepatocellular carcinoma; LTx, liver transplantation.

506 **Table 5**

Logistic regression	Uncorrected OR	p-value	Corrected OR	p-value
Normal transaminases at 26 weeks	2.57 (1.65 – 3.98)	<0.001	2.97 (1.66 – 5.33)	<0.001
Biochemical remission at 26 weeks	3.70 (2.07 – 6.61)	<0.001	3.62 (1.68 – 7.82)	0.001
Normal transaminases at 52 weeks	2.67 (1.64 – 4.37)	<0.001	2.45 (1.28 – 4.69)	0.007
Biochemical remission at 52 weeks	4.11 (2.21 – 7.64)	<0.001	4.34 (1.77 – 10.65)	0.001
Cox regression	**Uncorrected HR**	**p-value**	**Corrected HR**	**p-value**
Liver related death or LTx	0.33 (0.14 – 0.77)	0.01	0.47 (0.16 – 1.39)	0.17
All cause mortality	0.55 (0.25 – 1.14)	0.15	0.68 (0.26 – 1.79)	0.43

507 **Results of the validation cohort after multivariable logistic regression and Cox**
508 **regression for patients with a ≥80% AST decrease after 8 weeks of treatment.** In
509 all multivariable analyses we adjusted for institute, cirrhosis, AS-AIH, predniso(lo)ne
510 dose, use of maintenance therapy, AST at baseline and bilirubin at baseline. AS-AIH,
511 acute-severe AIH; AST, aspartate aminotransferase; HR, hazard ratio; OR, odds ratio;
512 LTx, liver transplantation.

513 **FIGURE LEGEND**

514

515 **Figure 1. Kaplan-Meier curve of liver related death or transplantation over time**
516 **in the discovery cohort.** Patients with an AST decrease of ≥80% are compared to
517 patients with an AST decrease <80% (log rank $p < 0.001$). AST, aspartate
518 aminotransferase; LTx, liver transplantation.

Figure